Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY REVIEW ARTICLE

Sialoadhesin – a macrophage-restricted marker of immunoregulation and inflammation

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doi:10.1111/imm.12042
Received 09 April 2012; revised 22 November 2012; accepted 22 November 2012.
This review discusses the macrophage receptor sialoadhesin with particular emphasis on its recently reported role in regulation of the adaptive immunity.
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Summary

Sialoadhesin (Sn, also known as Siglec-1 and CD169) is a macrophage-restricted cell surface receptor that is conserved across mammals. Sn is a member of the sialic acid-binding IgG-like lectin (Siglec) family of proteins characterized by affinity to specifically sialylated ligands, and under normal conditions is expressed on subsets of macrophages in secondary lymphoid tissues, such as lymph node and spleen. However, Sn-positive macrophages can also be found in a variety of pathological conditions, including (autoimmune) inflammatory infiltrates and tumours. Sn has been shown to contribute to sialylated pathogen uptake, antigen presentation and lymphocyte proliferation, and to influence both immunity and tolerance. This review presents Sn as a macrophage-specific marker of inflammation and immunoregulation with the potential to becoming an important biomarker for immunologically active macrophages and a target for therapy.

Keywords: regulatory T cell; Siglec; CD169; inflammation; tolerance; immunoregulation.

Introduction

In this review the structure, expression, regulation and function of the antigen CD169/Sialoadhesin (Sn) or Siglec-1 (originally dubbed sheep erythrocyte receptor) is evaluated. While the Siglec family, to which Sn belongs, has been the subject of numerous reviews,[1–9] only recently have researchers begun to discuss its role in the immune system.[10,11] This review seeks to consolidate our understanding of Sn as a member of the sialic acid binding, immunoglobulin-like lectins (Siglec) family and to surmise what is known concerning its structural and functional features, in particular with respect to its role in the immune system *in vivo*. We present Sn as a macrophage (Mϕ) -specific marker of inflammation and immunoregulation with the potential of becoming an important biomarker for immunologically active Mϕ and target for therapy.

Sialic acids, Sn and the Siglec family

Sialic acids are a family of sugars typically found attached to the exposed terminal position of cell surface and secreted glycan molecules.[12] The sialic acid binding receptor Sn was originally identified on a subset of Mϕ that were able to bind, but not phagocytose, sheep red blood cells in a sialic acid-dependent manner.[13,14] Sn was one of the first members of a subgroup within the immunoglobulin superfamily characterized by the use of sialylated glycans as ligands.[15,16]

Abbreviations:: CNS, central nervous system; CSF-1, colony-stimulating factor 1; EAE, experimental autoimmune encephalomyelitis; IFN, interferon; IL, interleukin; KO, knockout; LN, lymph node; LT, lymphotoxin; Mϕ, macrophage; MZ, marginal zone; MZM, marginal zone macrophage; MMM, marginal metallophilic macrophage; Neu5Ac, 5-*N* acetylated-neuraminic acid; SCS, subcapsular sinus; Siglec, sialic acid binding immunoglobulin-like lectin; Sn, sialoadhesin; Sn^+, sialoadhesin positive; Treg, regulatory T cell

Researchers went on to name this the Siglec family with criteria for inclusion being first the ability to bind sialylated glycans and second, significant sequence similarity within the N-terminal V-set and adjoining C2-sets domains.[3]

The Siglecs are divided into two groups, with the Sn-containing group displaying typical orthologues that can be identified in multiple mammalian species, while the CD33-related group, which is primarily found on myeloid and other innate immune cells, exhibits considerable inter-species variation.[2] Sn shows a high level of conservation between mouse and man, with considerable sequence similarity between the extracellular regions and comparatively little homology between the cytoplasmic domains. This observation, coupled to the lack of immunoreceptor tyrosine-based inhibitory motif in the cytoplasmic domain[4,17] as found on many of the CD33-like Siglecs as well as the Siglec CD22, has led to the speculation that Sn primarily serves extracellular functions, such as cell–cell and cell–matrix interactions.[18]

Sn was shown to be a 185 000 MW protein[19] with an extracellular region containing the extraordinary number of 17 immunoglobulin-like domains.[17] Analysis of Sn deletion mutants has established that the V-set terminal immunoglobulin-like domain is necessary and sufficient for sialic acid binding[20] and that this domain shows a preference for 5-*N* acetylated-neuraminic acid (Neu5Ac) which is α(2,3)-linked to preceding carbohydrates.[17,21] Use of synthetic sialosides revealed significant but low affinity for monovalent ligands (k_D in the range of 1 mM).[22] It is thought that simultaneous multivalent low-affinity associations will create sufficient high avidity and lead to biologically meaningful interactions of Sn for sialic acids on cells or microbial particles, which is supported by *in vitro* data demonstrating increased binding capacity of Sn with increasing saccharide content.[23] The crystal structure of Sn liganded to a model sialic acid in combination with mutagenesis studies has revealed the molecular basis for sialic acid recognition by Sn.[24]

It has been postulated that the extracellular region of Sn may function to extend the V-set domain away from the glycocalyx so as to enable interaction with ligands in a *trans* fashion.[25] However, even with the 17 immunoglobulin-like domains, extension of Sn above the glycocalyx may not always be guaranteed. A comparison of Sn activity in rat splenic and lymph node (LN) Mϕ has indicated that splenic Mϕ are masked considerably by endogenous sialylglycoconjugates through *cis*-type interactions which can be ablated by sialidase treatment, whereas LN Mϕ are relatively unaffected by masking.[26] Therefore the accessibility of Sn glycan binding can vary with location.

Sn expression and regulation

In human,[18] mouse,[27] rat[28] and pig[29] Sn is most abundantly expressed by Mϕ subsets occupying secondary lymphoid tissue (see Table 1). In all the species studied, Mϕ lining the subcapsular sinus (SCS) as well as the medulla of the LN highly express Sn, whereas splenic distribution varies between species (see Fig. 1). Marginal metallophilic Mϕ (MMM), a population that lines the marginal sinus at the periphery of the white pulp in rodents, express high levels of Sn. In rats marginal zone Mϕ (MZM) exhibit similar Sn levels to MMM, whereas in mice MZM expression is relatively lower. Both species demonstrate low levels of Sn expression in red pulp Mϕ. With differing splenic architecture in primates,[30] sialoadhesin-positive (Sn^+) Mϕ are located in a compartment found between the red pulp and the marginal zone (MZ) identified as the perifollicular zone.[31,32] In the absence of a marginal sinus and MMM in primate spleens, the Mϕ sheaths that line capillaries of the perifollicular zone are speculated to be equivalent to the MMM found in rodents.[31] Higher expression levels of Sn have been reported in human versus chimpanzee spleens and may result from the increased expression of Sn-ligand (Neu5Ac) in humans or from increased *cis*-inhibition due to sialic acid differences in chimpanzees.[32] Regardless of their equivalence, both the MZ and perifollicular zone environments do at least permit a close contact between Sn^+ Mϕ and the circulation and this could be relevant in the context of the physiological function of Sn. In pigs, splenic Sn expression is observed in the MZ and elliptical vessels,[29] which may be comparable to the periarterial Mϕ sheaths observed in humans.

In essence, the LN and the white pulp area of the spleen function as focal centres where microbial particles, antigen-presenting cells (which may carry antigen from the periphery or acquire antigen locally) and their corresponding antigen-specific lymphocytes engage and initiate

Table 1. Sialoadhesin expression in secondary lymphoid tissue

Tissue/Cell type	Rat	Human	Mouse	Pig
MMM	+	n/a	+	/
MZM	+	−	+/−	+
PALS	+/−	−	−	/
Splenic PFZM	n/a	+	n/a	n/a
PAMS	n/a	+	n/a	+[2]
Red pulp	+/−	+/−	+/−	−
LN supcapsular sinus	+	+[1]	+	+
LN medulla	+	+[1]	+	+

LN, lymph node; MMM, marginal metallophilic macrophage (Mϕ); MZM, marginal zone Mϕ; PALS, periarteriolar lymphoid sheath; PAMS, periarteriolar-associated Mϕ; PFZM, perifollicular zone Mϕ.
Key: n/a, not applicable; /, undetermined; −, undetected; +/−, low expression; + expression.
[1]Sinusoidal expression throughout LN.[99]
[2]Ellipsoidal vessels associated with Sialoadhesin expression, possibly equivalent to PAMS.[29]

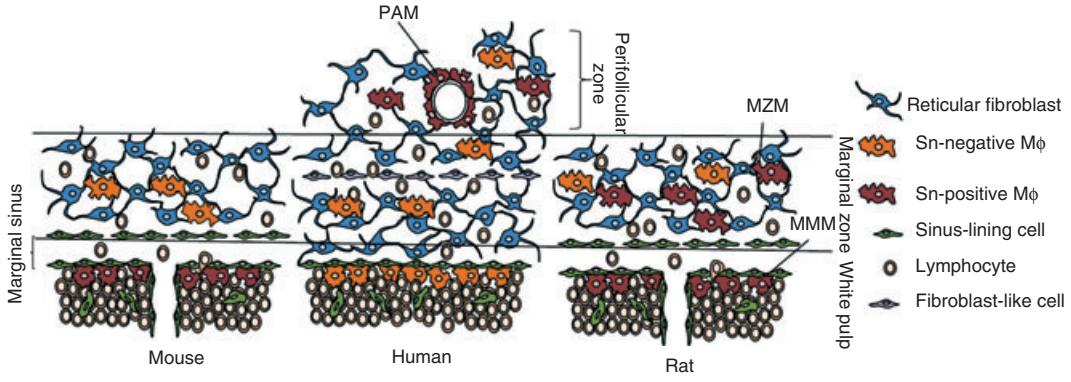

Figure 1. Comparison of the distribution of sialoadhesin-positive macrophages (Sn^+ Mϕ) in the splenic microenvironment. A framework of reticular fibroblasts (blue) forms the basis of the marginal zone and is continuous with the reticular fibroblasts in the red pulp and the sinus-lining cells (green) of the marginal sinus. In all species, Sn^+ Mϕ (red) are observed as a line of cells at the internal border of the white pulp (the so-called marginal metallophilic Mϕ (MMM). In rodents, the white pulp is separated from the marginal zone (MZ) by a marginal sinus, and in the case of the rat spleen, Sn^+ Mϕ are observed in the marginal zone. In humans, the marginal zone is absent of Sn^+ Mϕ, but the unique structure known as the perifollicular zone contains Sn^+ Mϕ both sporadically and clustered around arteriolae (known as periarteriolar-associated Mϕ (PAM). Figure adapted from ref. 30.

adaptive immune responses. The splenic marginal sinus/perifollicular region and the LN SCS are both transitional areas where cells and molecules enter the white pulp or LN parenchyma. MMM lining the marginal sinus of the spleen, where the blood enters the tissue, can therefore be considered an anatomically analogous position to Mϕ that form a rim under the LN SCS. Here, the afferent lymph enters the tissue, which is consistent with the idea that these cells and their Sn have a role in antigen capture/processing and in cell–cell communication.[33] This conserved positioning of Sn^+ Mϕ at the borders of lymphoid tissue and circulating fluids strongly suggests a function as a mediator of adaptive immunity.

Lower levels of Sn expression can be found on Mϕ in tissues other than lymphoid, with varying degrees of *cis*-masking shown to obscure apparent Sn levels.[26,34] Both mice and humans express quite high levels of Sn on resident bone marrow Mϕ, whereas in rats Sn is apparently absent from this population. Expression of Sn is consistently found in the liver across all species so far examined. Several other subsets of Mϕ have also been shown to express detectable levels of Sn, but this may again be somewhat variable among species.

Transgenic models have revealed the dependence of Sn^+ Mϕ populations upon certain mediators. Colony-stimulating factor 1 (CSF-1) is an important cytokine responsible for Mϕ maturation and migration, wherein the naturally occurring knockout (KO), the *op/op* mouse, exhibits variously depleted and diminished Mϕ subpopulations.[35] Sn-expressing Mϕ are absent from lymphoid tissue in the *op/op* mouse,[36] which can be restored following administration of CSF-1.[37] Interference with CSF-1 signalling has been shown to rapidly deplete Sn-expressing Mϕ in the spleen,[38] hence Sn Mϕ populations can be considered as developmentally regulated by CSF-1 production.

Lymphotoxin (LT) is responsible for generating and maintaining the architecture of secondary lymphoid organs, with its KO causing loss of structural organization.[39] Sn-expressing Mϕ are dependent upon the secretion of LT, with LT-α[40] and LT-β[41] deficiency leading to absence of this subset. From the findings that B-cell depletion affect Sn-expressing Mϕ in the SCS as well as the MZ,[42,43] B-cell LT expression was shown to be directly implicated.[42,44] Systemic treatment with LT antagonists replicates the phenotype of B-cell depletion, and in the SCS reveal Sn-expressing Mϕ to be replaced with a phenotype associated with medullary Mϕ.[45] Altogether these data demonstrate how intrinsic Sn Mϕ are to lymphoid tissue and their emergence is governed by its proper structuring.

As Sn is restricted to particular subsets of Mϕ the regulation of its expression has been of considerable interest. From the available data the regulation of Sn is broadly inflammatory and in particular is subject to type I and II interferon (IFN) induction. This strongly suggests a function in anti-viral and anti-bacterial activity; however, a role in general inflammation can be accounted for by its response to various inflammatory stimuli.

From the available data, Sn induction on peripheral blood mononuclear cells and various Mϕ subsets isolated from humans,[18,46,47] mice,[48] rats[49,50] and pigs[51] can be achieved by incubation with either type I or II IFN. The exception to this has been mice, for which, despite being the most extensively studied species for Sn, inducing factors have yet to be identified.[52,53] Rat,[49,50] mouse[48] and human Sn induction[18] has also been shown following tumour necrosis factor-α exposure, and additionally

ligation of human Toll-like receptors involved in viral and bacterial sensing[46] (which also induce IFN-α secretion) have been observed to increase expression. Interestingly, in the case of both mice and pigs an unidentified component of autologous serum has been shown to induce expression.[51,52] This dependence of expression upon circulating factors may well explain the observed changes in Sn expression on Mϕ populations following disruption of the blood–brain barrier[54] and afferent lymphatics.[55] The factors negatively regulating Sn are not as unified. T helper type 2 cytokines in mice[53] and both virus-sensing and bacteria-sensing Toll-like receptors in humans[46] have been shown to down-regulate expression, whereas interleukin-2 (IL-2) and IFN-γ demonstrated some inhibitory effects in rats.[56]

Anti-viral induction is consistent with the observed interaction between Sn and viral particles as well as with a functional role for Sn^+ Mϕ in mediating adaptive immune responses (see section: Functions of Sn and Sn+ Mϕ). The identification of Sn^+ Mϕ in a variety of inflammatory disease settings (see section: Sn in disease models) is also in line with regulation by inflammatory cytokines. However, less clear is the means of negatively regulating expression, and how both of these effects relate to Sn function *in vivo*.

Functions of Sn and Sn^+ Mϕ

The provision of Sn KO mice as well as data acquired from anti-Sn antibody-blocking studies has revealed specific functions of Sn during disease. Experiments in which Sn Mϕ populations have been depleted have demonstrated the activity of this cell subset.

Functions of Sn

Initial studies identifying Sn localization at the contact points of resident bone marrow Mϕ and erythoblastic islands suggested a role in haematopoiesis.[57] Although the development of Sn KO mice did not go on to demonstrate haematopoietic defects,[58] recent data is beginning to shed light on Sn^+ Mϕ function in the bone marrow (see section: Haematopoiesis).

T-cell interactions

The Sn KO mice exhibit decreased soluble IgM levels as well as subtle alterations in CD8 T-cell and various B-cell populations, which furthers the notion of Sn supporting immunoregulatory functions.[58] During experimental allergic encephalitis (EAE), Sn KO mice showed reduced disease severity along with reduced numbers of infiltrating T helper type 1 and type 17 cell in the central nervous system (CNS). Additionally, higher numbers of regulatory T (Treg) cells were observed in the CNS. *In vitro* proliferation of CD4 T cells using either Sn KO or wild-type CNS-infiltrating Mϕ as antigen-presenting cells showed lower Treg-cell proliferation using wild-type Mϕ, which could be ameliorated by blocking of Sn.[59] This study represents the first *in vitro* account of Sn Mϕ directly regulating T-cell behaviour. This pro-inflammatory effect of Sn is corroborated by other autoimmune studies in which Sn KO mice have exhibited reduced disease severity.[60,61] In a more artificial model of disease, Sn^+ Mϕ in the liver were observed to form clusters with CD4 and CD8 T cells during a successful graft-versus-leukaemia response. Following infusion of anti-Sn antibody, Mϕ–T-cell cluster formation, along with survival rate, were reduced. Additionally, isolated Sn Mϕ were shown to present MHC I and MHC II restricted peptides to CD8 and CD4 T cells, respectively.[62,63] Altogether these data show that Sn directly mediates the effects of the immune system that can be broadly classified as inflammatory. The exact nature of this effect, its mechanism and whether it is affected centrally (i.e. in lymphoid structures) or peripherally has yet to be determined.

Pathogen capture and cell–cell interaction

In vitro Sn has been shown to mediate binding to a variety of pathogens in a sialic acid-dependent manner.[64–67] This has led to the speculation that Sn primary function is to identify such structures and remove them from the lymph/blood directly, as evidenced by porcine and murine Sn-mediated endocytosis,[48,68] or via accessory receptors. In line with this, type I IFN regulation of Sn may serve to increase the recognition of sialic acid-displaying pathogens during infection. Based on the observation that Sn possesses an extensive, highly conserved extracellular domain that experiences considerably less *cis*-masking than other Siglecs, another hypothesis is that Sn mediates cell–cell interactions.[2] In line with this, Sn has been shown to display binding to various cell populations[18,69,70] with one study recording differential binding according to T-cell maturity in which authors reasoned that this assisted in T-cell homing to lymphoid tissue.[70] During EAE (see above) it was noted that Treg cells in the afflicted tissue displayed increased Sn ligand. *In vitro*, isolated Treg cells were then shown to proliferate in the absence/blocking of Sn expression on Mϕ that were acting as antigen-presenting cells, establishing the link between Sn-mediated interaction and lymphocyte behaviour.[59] Counter receptors for Sn including CD43,[71] MUC-1,[72] Mϕ galactose c-type lectin 1[73] and chimeric mannose receptor binding protein[74] contribute to our understanding that Sn can mediate interactions with various cell surface proteins. It has been speculated that to meet this requirement in identifying self-structures, Sn expression in humans is significantly higher than in

primates. The reasoning for this is based on the increase burden of the Sn ligand Neu5Ac in humans because of the loss of 5-*N* glycolyl neuraminic acid synthesis, leading to increased Sn expression to maintain efficient screening for self.[32]

Functions of Sn^+ Mϕ

Sn is expressed by Mϕ populations located in a variety of tissues; however, it is in the secondary lymphoid organs where populations exhibiting the highest expression levels are found (see previous section). Of these, Mϕ located in the SCS and medulla of the LN express the highest levels, followed by populations resident in various regions of the splenic MZ.

Activation of T and B cells and anti-pathogen immune response

To date, the functional roles Sn-expressing Mϕ in the LN have been best characterized. The SCS Mϕ border the lymphocyte-rich LN and flowing lymph, anatomically leading to the speculation that these resident cells perform a gate-keeping role, restricting access to the LN. This is exemplified by SCS Mϕ capture and secretion of IFN-α in response to neurotropic viral infection, preventing viral access to the CNS.[75] This demonstrates classical innate immune cell behaviour of pathogen scavenging and release of inflammatory mediators; however, further data illustrate Sn Mϕ direct action in supporting the adaptive immune response. Following subcutaneous injection of viral particles, SCS Mϕ have been shown to prevent the systemic dissemination of virus through its capture followed by initiation of adaptive immunity directly by presentation antigen to B cells.[76] Similarly, SCS Mϕ-mediated capture, processing and presentation of antigenic material to inducible natural killer T cells,[77] CD8 T cells[78] and B cells[42] has demonstrated this connection between a classical scavenging role and engaging adaptive immunity.

The spleen similarly locates Sn^+ Mϕ in the vicinity of lymphocyte-rich tissue and flowing fluid (in this case blood rather than lymph). Because of the difficulty of isolation or selected depletion of either marginal metallophilic Mϕ and marginal zone Mϕ subsets (the splenic Mϕ populations that express high Sn depending upon species) identifying specific roles for these subsets has so far proven troublesome.[79] Depletion of MZ Mϕ (MMM + MZM) via clodronate liposomes[80] or use of transgenics[81] and exposure to apoptotic cells has revealed the importance of MZ Mϕ debris clearance and maintenance of tolerance, with their absence leading to autoimmunity. The presence of MZ Mϕ was also required in generating a CD8 T-cell response to intravenous viral infection.[82] This study showed that targeting of antigen specifically to Sn^+ Mϕ promoted a CD8 T-cell response, involving cross-presentation with CD8 dendritic cells but not eliminating a possible direct activation role for Sn^+ Mϕ. More recently, an effective humoral response to vesicular stomatitis virus was shown to involve Sn^+ Mϕ capture and subsequent enforced replication, thought to enrich antigen load and enhance immunity.[83] The SCS Mϕ were similarly observed as repositories for viral replication in a response to vesicular stomatitis virus challenge in the LN, which is thought to enhance immunity.[75] These data all point to the specialized role that Sn^+ Mϕ play as scavengers and enforcers of adaptive immunity.

From these findings that MZ and SCS Mϕ participate in the induction of an adaptive immune response, targeting antigen to Sn^+ Mϕ has been explored as a means of inducing immunity. The delivery of antigen to Sn using a monoclonal antibody has been shown to strongly inhibit tumour growth in mice[82] whereas a humoral response and increased reactivity of peripheral blood mononuclear cells was observed in pigs.[84] Without specifically targeting Sn^+ Mϕ, an efficient anti-tumour response was mounted following subcutaneous injection of necrotic tumour cells. This effect was abolished by selective depletion of Sn^+ Mϕ in LN, illustrating the importance of this subset in vaccination.[78] Coupled to the data concerning the fact that MZ Mϕ maintain self-tolerance following exposure to apoptotic material, it is clear that Sn^+ Mϕ represent important links in the induction of tolerance and in tolerance (i.e. vaccination).

Interestingly, this close relationship between Sn-expressing Mϕ and lymphocytes presents a potential opportunity for pathogen transmission. SCS Mϕ capture of *Toxoplasma gondii* results in T-cell recruitment and subsequent transfer of parasite from Mϕ to T cell, which can then promote host invasion of the pathogen by trafficking.[85]

Haematopoiesis

Finally, Sn^+ resident bone marrow Mϕ have been shown to promote the retention of haematopoietic stem cells, with their ablation inducing their egress into the circulation.[86] This result represents the first functional data concerning Sn-expressing Mϕ in the bone marrow, and although the precise nature of this effect has yet to be elucidated, it confirms the significance of this cell type external to the lymphoid environment.

Sn in disease models

A variety of disease models have been characterized in which Sn^+ Mϕ are involved in the immune response. In addition to the data below, Sn^+ Mϕ have also been found in models of chronic rejection,[87] neointima injury,[88] cerebral vasculature injury[89] and atherosclerosis.[90]

Cancer

Macrophages play an important role in the pathophysiology of cancer[91] and cancer models have provided direct insights into the function of Sn. In murine graft-versus-leukaemia reactivity models, increased Sn expression in liver and spleen correlated with lymphoma stasis/regression.[62,92,93] Adoptive immunotherapy showed donor lymphocytes clustered with Sn^+ Mϕ and isolated Mϕ able to act as a professional antigen-presenting cell by presenting antigen to CD4 and CD8 T cells.[62,93] Furthermore, *in vivo* administration of anti-Sn monoclonal antibody resulted in reduced accumulation of CD8 T cells and cluster formation, as well as reduced survival rates. *In vitro* Sn blocking showed reduced proliferation of cytotoxic T lymphocytes in the presence of Sn^+ Mϕ pulsed with antigen.[63] These Sn^+ Mϕ were later shown to express increased levels of CD40, a receptor that is involved in T-cell priming, activation and differentiation as well as in the mediation of Mϕ effector functions, suggesting that Sn^+ Mϕ activate recruited T cells and hence promote a tumoricidal response.[94]

Analysis of rat tumours has shown Sn^+ Mϕ to be present in hepatic metastatic carcinoma[95] and adenocarcinoma of the prostate.[96] In prostate adenocarcinoma the number of Sn^+ Mϕ showed a strong positive correlation with tumour apoptosis and strong negative correlation with tumour growth. As in mice, rat Sn expression was found to be concomitant with lymphocyte recruitment. In a rat study of xenograft transplanted tumours, Sn-expressing Mϕ were found to infiltrate the intratumoral environment depending upon the secretion of monocyte chemoattractant protein-1 by the xenograft,[97] which was confirmed by the rapid recruitment of Sn^+ Mϕ following monocyte chemoattractant protein-1 administration.[98]

Clinically, increased Sn expression has been recorded in splenic marginal cell lymphoma[99] as well as in Mϕ infiltrates of MUC-1-positive breast carcinoma.[72] These data make it clear that Sn^+ Mϕ are immunologically important players in the host response to cancer. With the identification of Sn^+ Mϕ as successful targets for tumour vaccination (see section: Functions of Sn and Sn+ Mϕ), teasing out the specific biological effects of Sn-expressing Mϕ during cancer may prove valuable for the understanding and management of this disease.

Autoimmune inflammation

Since the identification of Sn, autoimmune diseases have been probed for their expression of this antigen. The following summarizes models that have exhibited Sn-expressing Mϕ. Recently the increasing amounts of data supporting a role for Sn^+ Mϕ in adaptive immunity point to a functional significance of this Mϕ subset in the models outlined below.

Arthritis

In the rat, accumulation of Sn^+ Mϕ into the arthritic synovium and joint space has been shown to occur within a day following induction and along with lymphocyte influx.[100–102] Following arthritic induction, increased Sn expression in the LN SCS, splenic MZ and red pulp has been found, which may be a reflection of the increased serum levels of relevant inflammatory cytokines[103] (see section: Concluding remarks). Similarly, a systemic increase in tumour necrosis factor-α has been observed after arthritis induced by intraperitoneal injection with streptococcal cell wall fragments in which elevated Sn expression was found on isolated peritoneal Mϕ.[104] Treatment with clodronate-laden small unilamellar vesicles has been shown to reduce overall Mϕ burden (including Sn^+ Mϕ) and arthritis severity,[105] although whether this effect resulted specifically from the Sn^+ Mϕ subset was undetermined.

It is yet to be established whether Sn^+ Mϕ are complicit in disease regulation/progression or are simply by-products of the inflammatory setting. With the identification of Mϕ expressing high levels of Sn in the joints of patients with rheumatoid arthritis,[18] this subset are once again conspicuous in a disease process.

Glomerulonephritis

In rats, Sn expression in Mϕ populations has often been used as an indicator of Mϕ maturity/activation during nephritis.[106–112] Anti-glomerular basement membrane models of nephrotoxic nephritis have shown reduction in Sn^+ Mϕ numbers following anti-inflammatory treatment (IL-4,[113] IL-6,[114] IL-11,[111] high dose ANG II type 1 receptor blockade[115] and infusion of bone-marrow-derived Mϕ transduced to express IL-10[116]). In humans, analysis of kidney tissue from patients with glomerulonephritis has shown that Sn^+ Mϕ accumulation correlated with glomerular injury, with expression dropping following glucocorticoid therapy.[117] As with arthritis, a functional role for Sn^+ Mϕ glomerular nephritis has not been assigned. It is possible that expression is passive and a consequence of the inflammatory milieu rather than participating as a mediator of the adaptive immune response.

CNS inflammation

The role of Sn Mϕ has also been studied in autoimmunity models, with several data from murine experimental disease models providing further evidence for an immunoregulatory role of Sn. The Sn KO mice exhibit reduced disease severity in experimental autoimmune uveoretinitis,[61] and myelin degeneration models,[60,118] which correlated with reduced lymphocyte invasion. Moreover, in a mouse myelin oligodendrocyte glycoprotein-induced EAE

disease severity was lower in Sn KO whereas leucocyte recruitment was shown to be unaffected. During EAE, increased Treg cells and decreased effector T-cell numbers were detected in the CNS of KO compared with wild-type mice. *In vitro*, Sn expression was shown to directly influence Treg-cell proliferative responses (see section: Functions of Sn and Sn+ Mϕ).[59]

In rat studies, during experimental autoimmune disease induction it was found that monocytes isolated from rat strains that were more susceptible to experimental autoimmune disease expressed considerably higher levels of Sn following culture than those from less susceptible strains.[119] Similar to mice, induction of EAE resulted in the recruitment of Sn^+ Mϕ throughout the CNS parenchyma and meninges[120] and their presence is also recorded in an experimental autoimmune pigment epithelial protein-produced uveitis model.[121] Once again lymphocyte recruitment was positively correlated with increased Sn; however, no causal relationship has been shown. It was noted that in EAE-susceptible rat strains the induction of Sn on monocytes was strongly dependent on the presence of T cells. It is unclear if Sn^+ Mϕ are functionally significant in disease pathogenesis; however, the mutual relationship between T cells and Sn-expressing Mϕ is yet again evident, implicating this subset in adaptive immunity.

Role of Sn Mϕ in disease settings

From the available data, Sn KO and inhibition diminish the adaptive immune response in the above models. This is not surprising given that Sn^+ Mϕ border the lymphocyte-rich regions of the body and promote immunity (see section: Functions of Sn and Sn+ Mϕ). From this observation it is tempting to speculate that the purpose of Sn is to somehow promote adaptive immune responses as mediated by Mϕ. Although the studies on rheumatoid arthritis and glomerulonephritis do not establish a function for Sn, it is feasible that the diminished immune response observed in the KO and inhibition of CNS inflammation and cancer models would similarly be witnessed during these settings.

Alongside delineating the precise action of Sn, i.e. is it a self-recognition receptor that mediates cell recruitment/interaction, remains the question are these responses a consequence of Sn exerting its influence centrally in lymphoid tissue-resident Mϕ or does Sn also mediate events at the sites of disease. The functional performance of Sn specifically in lymphoid tissue has yet to be assessed; however, given the alterations of IgM levels and lymphocyte numbers in Sn KO mice compared with wild-type mice[58] there is evidence of its significance in steady state, which is presumably lymphoid-restricted activity. At the other end, evidence supporting Sn activity outside the lymphoid compartment (and at the site of disease) comes from the recent finding that T-cell proliferation is directly influenced by Sn expression[59] (see section: Functions of Sn and Sn+ Mϕ) which provides a functional purpose behind its expression in diseased tissue as a regulator of T cells. However, the possibility remains that Sn expression is simply a passive response to inflammatory stimuli. Given the conserved expression of this marker across species in related disease processes as well as its functional significance as established by KO models and blocking, it is apparent that Sn is a critical receptor implicated in the adaptive immune response during a variety of disease settings.

Concluding remarks

In the 25 years since its identification, researchers have managed to elucidate many structural and functional properties of Sn. Displaying a confinement in its expression to Mϕ in transition regions between lymphoid tissue and flowing lymph/blood, Sn presents itself as at least one of the most restricted of Mϕ markers. Sn is conserved across a host of species, and possesses an extended structure able to bind to extracellular sialic acid-containing carbohydrates. This recognition of host-derived sialic acid is anticipated to enable Mϕ to interact with various components of the immune system to coordinate host immune responses in an effective fashion.

The wealth of data collected show that Sn^+ Mϕ are implicated in a host of diseases, according it immediate value as a means of monitoring inflammatory processes. The recent data concerning Sn^+ Mϕ function make this cell population increasingly attractive as targets to manipulate the adaptive immune response. This is no more strikingly demonstrated than the application of Sn targeting to modulate immunity and tolerance, which has implications for vaccine development.

Future investigation should further clarify the role of Sn and this will also provide insight into the specialized function of the defined Mϕ subset in lymphoid organs that expresses this unique Mϕ receptor.

Acknowledgements

ASGO'N would like to thank PR Crocker for critical review of the manuscript and suggestions. ASGO'N is supported by a Harris Studentship.

Authors contribution

ASGO'N conceived of the review and wrote the manuscript. TKvdB and GEDM revised drafts.

Disclosure

The authors declare that there are no conflicts of interest.

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